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EFFECT OF DOUM (*Hyphaene thebaica)*® ON RESPONSE OF RABBIT SPERMATOZOA TO HYPO-OSMOTIC SWOLLEN TEST

Safaa A. Barakat *and Rowida M. Riad**
**- Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.*
***- Animal Reproduction Research Institute, Agricultural Research Center, El-Haram, Giza, Egypt.*
***E.mail of corresponding author**: safaa_ataya@yahoo.com.*

Eighteen sexually mature Hy-Plus rabbit bucks of nine months old were used in the present study. The study was designed to evaluate grade and percentages of progressive sperm motility, in addition to percentages of spermatozoa with swollen heads or coiled tails of diluted semen supplemented with different levels of Doum aqua's extract as responded to hypo-osmotic solution, during incubation at 37 ^{o}C for up to 60 minutes. Semen was collected artificially and samples showing sperm motility less than 70% were removed. Accepted semen samples were pooled and divided into two parts. The first part was diluted at 300 mOsm/ L (normal osmolarity) while the other was diluted at 100 mOsm/L (hypo-osmotic pressure). Each part of diluted semen was subdivided into four parts. The 1st group was kept untreated as a control, and Doum aqua's extract were added to 2nd, 3rd and 4th groups at concentrations of 1, 2 or 3 ml/ 100 ml diluted semen, respectively.

***Results obtained indicated that**, sperm motility in solutions with different osmolarities (300 or 100 mOsm/L) were significantly (P ≤ 0.01) high, while the percentages of spermatozoa with coiled tails or swollen heads were significantly (P ≤ 0.01) lower at level of 300 mOsm/L (normal osmolarity) than 100 mOsm/L (osmotic stress), during incubation at 37 ^{o}C for up to 60 minutes in rabbit semen groups.*

Advancement of incubation time up to 60 minutes of diluted rabbit semen in solutions with different osmolarities significantly (P ≤ 0.01) decreased grade of sperm motility and increased advanced sperm motility percentage spermatozoa with swollen heads and coiled tails, respectively. Progressive sperm grades and percentages and percentages of spermatozoa with swollen heads or coiled tails in both osmolarities used were significantly (P ≤ 0.01) arranged in descending order as recorded by diluted semen added with 3, 2, 1 and 0 mg Doum aqua's extract/ 100 ml diluted semen, respectively.

Adding 3 ml Doum aqua's extract/ 100 ml diluted semen insignificantly increased grade and percentages of sperm motility and spermatozoa with swollen heads, compared with 2 ml of the extract.

***Conclusively**, it could be concluded that, adding diluted rabbit semen with aqua's extract of Doum improved semen quality, and prolonged spermatozoa storagability, during semen conservation at incubation condition.*

Key words: Rabbit, semen, Doum, Hyphaene Thebaica, incubation, osmolarity.

It is considered that one single buck is affecting the fertility and prolificacy of about one hundred does, if artificial insemination is applied in a rabbitries, (Alvarino, 2000). So, reliable evaluation of semen and fertilizing ability of bucks is of great importance for successfulness of AI technique.

Studying sperm membrane functional status is of particular importance since an intact and functionally active membrane are required for metabolism, capacitation, acrosome reaction, attachment and penetration of the oocyte (Jeyendran *et al.*, 1984 ; Zeidan *et al.*, 2005). Thus assessment of the sperm membrane functional status appears to be a significant marker for the fertilizing capacity of spermatozoa (Jeyendran *et al.*, 1984; Zaneveled *et al.*, 1990).

No one single and simple parameter for semen evaluation can be used as reliable predictor of sperm fertilizing ability (Daader and Seleem, 2005). The hypo-osmotic swollen test (HOS-test) was used to evaluate response to hypo osmotic conditions in human (Jeyendran *et al.*, 1984), and rabbit spermatozoa (Daader and Seleem, 2005 ; Zeidan *et al.*, 2005). Some studies have shown that HOS-test is more reliable in assessing the outcome of *in vitro* fertilization than other semen parameters (Zaneveld *et al.*, 1990).

Hussein *et al*. (2011) studied the chemical composition and physicochemical properties of Doum, Perry leaves and Carob. The antioxidant activities of these extracts were investigated by scavenging of 1, 1 diphenyl-2-picrylehydrozyl (DPPH) radicals. The proliferation inhibition activities on some types of bacteria and yeast were also measured for evaluating the antimicrobial activity of Doum tea infusions and their blends. The results showed that carob was characterized by its high protein content. The highest percentage of fiber was found in Doum sample. The levels of most elements were high. The highest value of the content of total soluble solids (TSS) was found in the tea infusions of Doum.

Some biologically effective components of the medicinal herb possess anti-oxidative/ free radical scavenging properties have been reported to improve sperm functions *in vitro* and *in vivo* (Zheng and Zhang, 1997, Suzuki *et al.*, 2003 ; Zhang *et al*., 2006). The capacity of sperm fertilization is principally dependent on sperm motility and sperm membrane integrity and fertilization will be impaired if they are damaged. Nitric oxide (NO) is a biologically active free radical and also an important intracellular and intercellular messenger, which is generated in mammalian cells from L-arginine by family of Nitric oxide synthasas (NOS) (Marletta, 1993). Nitric oxide is beneficial to sperm motility as it was indicated to play a significant role in modulation of sperm functions (Lewis *et al.*, 1996) and acrosomal reaction (Revelli *et al*., 1999). Recently, Doum have been shown to increase human sperm motility *in vitro* (Chen *et al.*, 2001 , Zhang *et al*., 2006). Doum has been widely reported to stimulate the activity of NOS in a variety of cells and tissues (Scott *et al*., 2001 ; Bai *et al*., 2003 and 2004).

Therefore, the present study was designed to evaluate the response of spermatozoa of Hy-Plus rabbit bucks to (HOS-test) as affected by adding Doum aqua's extract to diluted semen, during incubation at 37 ^{o}C for up to 60 minutes.

MATERIALS AND METHODS

The field part of the present study was carried out in an industrial Rabbitry, at Sakarah city, Giza province, Egypt; the laboratory work was conducted in Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.

Eighteen sexual mature Hy-Plus rabbit bucks of nine months old and 4180 ± 40 gm body weight were used in the present work. All animals were fed *ad libitum* a commercial pelleted diet according to **NRC (1994)** recommendations. The ingredients and chemical composition of the pelleted ration fed to rabbits, during the collection period is shown in Table 1**.**

Animals were healthy and clinically free of external and internal parasites. The animals were kept under the same managerial and hygienic conditions.

The present work was planned to estimate the response of spermatozoa in semen ejaculated by Hy-Plus rabbit bucks to hypo-osmotic shock (swollen or coiled) (HOS-test) as affected by aqueous extracts of Doum at levels 0, 1, 2 or 3 ml/ 100 ml diluted rabbit semen, during incubation at 37 ^{o}C for up to 60 minutes. The HOS-test represented by each of grade and percentages of progressive sperm motility, in addition to percentages of spermatozoa with swollen heads or coiled tails.

Table 1. Ingredients and chemical composition of the pellet ration fed to rabbits, during the experimental.

Ingredient	**(%)**	**Vitamins & Minerals premix per Kilogram.**	
Clover hay	30.00	Vit.A (IU)	10,000
Wheat bran	26.20	Vit.D3 (IU)	2000
Barley grain	23.00	Vit.E (IU)	5000
Soybean meal (44%)	16.00	Vit.K (IU)	2
Molasses	3.00	Vit.B1 (IU)	2
Lime stone	1.00	Vit.B2 (IU)	4
Sodium chloride	0.50	Vit.B6 (IU)	3
Vitamins & Mineral Premix	0.30	Vit.B12 (IU)	0.02
Total	**100**	Biotin (mg)	0.2
Calculated chemical composition **		Choline (mg)	1200
Crudeprotein (CP)%	16.72	Niacine (mg)	40
Ether extract (EE)%	2.95	Zn. (mg)	60
Crude fiber (CF)%	13.07	Cu. (mg)	0.1
Digestible energy (Kcal/Kg)	2490	Mn. (mg)	62
		Fe. (mg)	40
		Folic acid (mg)	1
		Pantothenic acid (mg)	15

** Calculated according to **NRC (1994)** for rabbits. لايوجد ذلك انما ١٩٧٧

Aqueous extracts of Doum were prepared by transfer of 10 grams of the Doum powder to sterile wide-mouthed screw-capped bottles; 100 ml of sterile de-ionized distilled water was added to the powder samples and allowed to be soaked for 3 hours. The mixture was then centrifuged at 1000 rpm for half an hour. The supernatants were filtered through a 0.45 µm membrane (Kim *et al*., 2002).

Semen was collected artificially twice a week for 3 months using an artificial vagina as described by Boiti *et al.* (2005). The ejaculated semen was evaluated microscopically and only ejaculates that showed advanced motility ≥ 70% were pooled and diluted with saline solution (sodium

chloride NaCL 0.9 gm, egg yolk 5 ml, 50000 IU sodium penicillin and 50000 µg streptomycin sulphate/ 100 ml sterilized distilled water) to produce a final dilution rate of 1 semen: 10 diluent, and at osmolarities 100 or 300 mOsm/L (Daader and Seleem, 2005 ; Zeidan *et al.*, 2005).The diluted semen in different osmolarities were incubated at 37^{o}C for up to 60 minutes. The final osmolarity of the test solutions measured by freezing point depression was modified from 300 mOsm/L. (normal osmolarity) to 100 mOsm/L (stress or hypo-osmolarity) via serial dilutions to obtain hypo-osmotic solution.

The diluted semen was divided into four portions and supplemented with the different concentrations of Doum extract (0, 1, 2 and 3 ml/ 100 ml diluents). All treated diluted semen samples were incubated at 37 ^{o}C for up to 60 minutes.

Grades and percentages of advanced sperm motility and spermatozoa with coiled tail and swollen head were estimated at different times (0, 15, 30, 45, and 60 minutes) of incubation at 37^{o}C. Grade of the progressive sperm motility was graded according to Zavos *et al.* (1994) as follows: Grade 1, oscillating movement but stationary, Grade 2, slow movement with no fixed direction, Grade 3, slow progressive movement and Grade 4, fast progressive movement. Sperm swelling was assessed by placing 15µl of well-mixed sample on a warm slide (37^{o}C). Slides were stained with eosin-nigrosin mixture and covered with a cover glass before, being observed under a phase contrast microscope at x1000. Two hundred spermatozoa per slide were counted and the percentage of swelling/coiling was determined (number of spermatozoa with swollen/coiled tails divided by the total number of spermatozoa counted multiplied by 100). The proportion of coiled/swollen spermatozoa from a control sample (300mOsm/L) was subtracted from the calculations (Vazquez *et al.*, 1997). The response of spermatozoa to hypo-osmotic shock test was carried out as studied by Daader and Seleem (2005) and Zeidan *et al.* (2005).

Data were statistically analyzed by analysis of variance according to SAS (2001). Percentage values were transformed to arcsine before being statistically analyzed. Duncan's multiple range test (Duncan, 1955) was used to test the significance differences among means. Kindling rates were analyzed using the Contingency Tables according to Everitt (1977).

RESULTS AND DISCUSSION

Progressive sperm motility:

Data presented in Tables 2 & 3 showed that, the highest progressive sperm motility grades and percentages in both osmolarities used, during all

Table 2. Grades of progressive sperm motility of Hy-Plus rabbit semen diluted with different levels of Doum as response to hypo-osmotic solution, during incubation at 37 °C for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsm/L)	Level of aqueous extracts of Doum **(ml/ 100 ml diluted semen)**				**Overall means**
		0.0 (Control)	**1 (T_1)**	**2 (T_2)**	**3 (T_3)**	
0	300	3.07±0.11	3.40 ± 0.20	3.81 ± 0.22	3.96 ± 0.23	3.56± 0.18
	100	2.85±0.10	3.03 ± 0.17	3.58 ± 0.21	3.80 ± 0.20	3.32± 0.14
Means		**2.96±0.09 c**	**3.22± 0.14 b**	**3.70± 0.18 a**	**3.88± 0.20 a**	**3.44±0.11 a**
15	300	3.00±0.11	3.42 ± 0.15	3.85 ± 0.24	3.89± 0.26	3.54± 0.16 a
	100	1.99±0.11	2.60 ± 0.10	3.48 ± 0.18	3.68± 0.19	2.94± 0.13 b
Means		**2.50±0.08 c**	**3.01± 0.11 b**	**3.67± 0.20 a**	**3.79± 0.21 a**	**3.24± 0.12 a**
30	300	2.91±0.10	3.30 ± 0.12	3.79 ± 0.21	3.84±0.24	3.46± 0.18 a
	100	1.24±0.09	2.12 ± 0.12	3.10 ± 0.14	3.21± 0.14	2.42± 0.11 b
Means		**2.08±0.07 c**	**2.71± 0.10 b**	**3.45± 0.15 a**	**3.53± 0.16 a**	**2.94± 0.13 b**
45	300	2.73±0.10	3.27 ± 0.11	3.71 ± 0.15	3.82 ± 0.18	3.38±0.13 a
	100	0.75±0.06	1.72± 0.08	2.82 ± 0.07	2.93± 0.10	2.06± 0.9 b
Means		**1.74±0.08 c**	**2.50± 0.09 b**	**3.27± 0.09 a**	**3.38± 0.12 a**	**2.72± 0.11 b**
60	300	2.49±0.11	3.21 ± 0.11	3.63 ± 0.14	3.78 ± 0.13	3.28±0.10 a
	100	0.32±0.02	1.43± 0.05	2.61 ± 0.08	2.78± 0.10	1.79±0.04 b
Means		**1.41±0.05 c**	**2.32± 0.06 b**	**3.12± 0.07 a**	**3.28± 0.09 a**	**2.53±0.06 c**
Overall means		**2.14±0.05 c**	**2.75± 0.07 b**	**3.44± 0.10 a**	**3.57± 0.11 a**	**2.98±0.08**
Storagability (%)	300	81.11±1.86	94.41±2.01	95.28±2.08	95.45±2.04	91.56±2.54 a
	100	11.23±0.51	47.19±1.12	72.91±1.34	73.16±1.21	51.12±0.91 b
Means		**46.17±1.37 c**	**70.80±1.82 b**	**84.10±1.91 a**	**84.31±1.99 a**	**71.34±1.13**

Means within the same row (a, b, c) or within the same column (A, B, C) bearing different letter superscripts are significantly ($P \leq 0.05$ or 0.01) different.

incubation times were recorded in diluted semen with 3, 2, 1 then 0 ml aqueous extract of Doum /100 ml, respectively. The effect of semen treated with Doum on progressive sperm motility grades and percentages was highly significant ($P \leq 0.01$).

Grades and percentages of progressive sperm motility of the four semen samples used were significantly ($P \leq 0.01$) lower in hypo-osmotic solution (100 mOsm/L) than normal osmotic solution (300mOsm/L). These results are in agreement with those obtained by Daader and Seleem (2005) and Zeidan *el al.* (2005).

The advancement of incubation time at 37^0C for up to 60 minutes decreased significantly ($P \leq 0.01$) the grades and percentages of progressive sperm motility in all rabbit semen studied and in both osmolarities used. These results are comparable with those obtained by Daader and Seleem (2005) and Zeidan *et al.* (2005).

Table 3. Percentage of progressive sperm motility of Hy-Plus rabbit semen diluted with different levels of Doum as responded to hypo-osmotic solution, during incubation at 37 ^{o}C for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsm/L)	Levels of aqueous extracts of Doum (ml/ 100 ml diluted semen)				Overall means
		0.0 (Control)	1 (T_1)	2 (T_2)	3 (T_3)	
0	300	55.21±1.61	59.18± 1.73	61.55 ± 1.84	63.11 ± 1.97	59.76± 1.81 ^{a}
	100	48.15±1.45	51.24 ± 1.51	55.16 ± 1.66	57.03± 1.74	52.90± 2.11 ^{b}
Means		**51.68±2.01** ^{c}	**55.21± 1.94** ^{bc}	**58.34± 1.99** ^{ab}	**60.07± 2.05** ^{a}	**56.33±1.94** ^{a}
15	300	55.01±1.65	59.14± 1.58	61.70 ± 1.78	63.12± 1.89	59.74±1.89^{a}
	100	33.14±1.31	41.17 ± 1.46	45.12 ± 1.55	47.06 ± 1.72	41.62±1.75^{b}
Means		**44.08±1.77** ^{c}	**50.16± 1.88**^{b}	**53.41± 2.03** ^{ab}	**55.09± 1.99** ^{a}	**50.69±1.89** ^{b}
30	300	53.92±1.24	58.84 ± 1.31	61.01 ± 1.57	62.68 ± 1.81	59.11±1.75^{a}
	100	19.13±0.91	31.14 ± 0.99	36.42 ± 1.17	38.91± 1.29	31.40±1.32^{b}
Means		**36.53±0.98** ^{d}	**44.99± 0.87**^{c}	**48.72± 0.93** ^{b}	**50.80± 1.02** ^{a}	**45.26±1.27** ^{c}
45	300	53.15±1.14	58.61 ± 1.14	60.87 ± 1.27	62.60 ± 1.23	58.81±1.50^{a}
	100	08.71±0.08	22.46 ± 1.11	28.94 ± 1.11	31.01± 1.03	22.78±1.44^{b}
Means		**30.93±0.88** ^{c}	**40.54± 0.95**^{b}	**44.91± 1.14** ^{a}	**46.81± 1.32** ^{a}	**40.80±1.14** ^{d}
60	300	51.11±0.91	57.09 ± 1.31	60.34 ± 1.42	62.39 ± 1.38	57.73±1.32^{a}
	100	03.51±0.36	17.99± 0.41	25.31 ± 0.49	27.93 ± 0.48	18.69± 0.65^{b}
Means		**27.31±0.84** ^{d}	**37.54± 1.02**^{c}	**42.83± 1.17** ^{b}	**45.16± 1.08** ^{a}	**38.21±0.83** ^{e}
Overall means		**38.11±0.95** ^{d}	**45.69± 1.03**^{c}	**49.64± 1.12**^{b}	**51.59± 1.19** ^{a}	**46.26±1.01**
Storagability (%)	300	92.57±2.02	96.47±2.26	98.03±2.77	98.86±2.84	96.48±2.45 ^{a}
	100	07.29±0.21	35.11±0.66	45.88±0.81	48.97±1.15	34.31±0.58 ^{b}
Overall means		**49.93±1.53** ^{c}	**65.79±1.77**^{b}	**71.96±1.83** ^{a}	**73.92±1.92** ^{a}	**65.40±1.15**

Means within the same row (a, b, c, d) or within the same column (A, B, C, D, E) bearing different letter superscripts are significantly (P ≤0.05 or 0.01) different.

Percentages of sperm storagability were arranged (P ≤ 0.01) descendingly as obtained by semen treated with 3, 2, 1 then 0 ml aqueous extract of Doum 100 ml diluted semen, respectively.

It is interested to notice that, the grades and percentages of progressive motility of spermatozoa in solution at 300 mOsm/L were superior (P ≤ 0.01) from those in solution at 100 mOsm/L during different times of semen preservation at incubation condition.

Spermatozoa with swollen head or coiled tails:

Data presented in Tables 4 & 5 showed that, osmolarity tested solution (100 mOsm/L, hypo-osmotic pressure) recorded the highest (P ≤ 0.01) percentages of spermatozoa with swollen heads or coiled tails, during incubation at 37 ^{0}C for up to 60 minutes due to supplementation of 3, 2 and 1 ml aqueous extract of Doum/ 100 ml diluted semen, respectively. An adverse results were obtained by using normal osmolarity solution (300 mOsm/L).

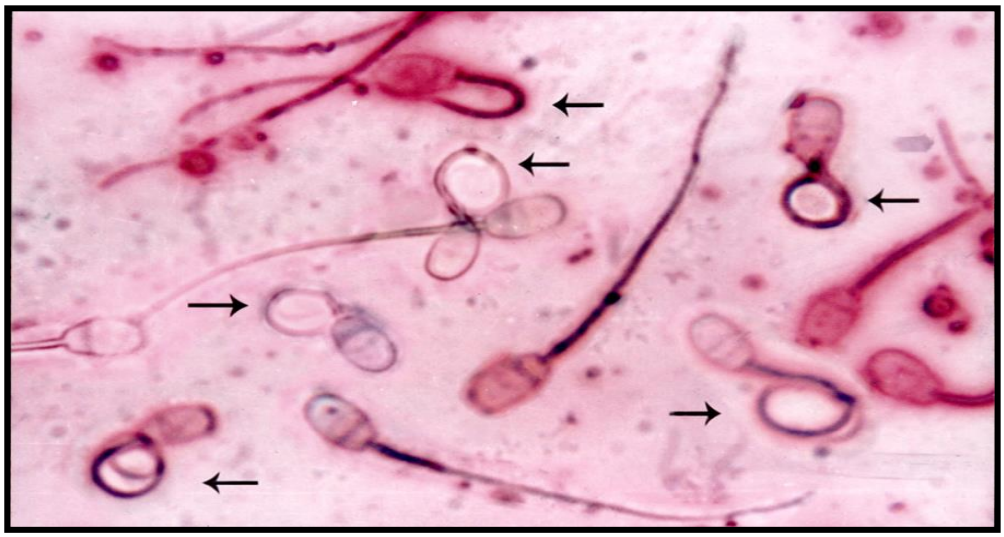

Figure 1. Micrograph of the response of Hy-Plus rabbit spermatozoa to the hypo-osmotic swollen test (HOS-Test) (black arrows indicated spermatozoa with coiled tails).

Table 4. Percentage of spermatozoa with swelling heads of Hy-Plus rabbit semen diluted with different levels of Doum as responses to hypo-osmotic solution, during incubation at 37 ^{o}C for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsm/L)	Level of aqueous extracts of Doum (ml/ 100 ml diluted semen)				Overall mean
		0.0 (Control)	1 (T_1)	2 (T_2)	3 (T_3)	
0	300	08.31±0.44	07.54± 0.41	07.61± 0.41	05.39±0.21	07.21±0.29^{b}
	100	09.72±0.46	12.11±0.52	12.27± 0.48	12.34±0.46	11.61± 0.39^{a}
Means		**09.02±0.41^{b}**	**09.83±0.45^{ab}**	**09.94±0.44^{a}**	**08.87±0.51^{b}**	**9.41±0.31 ^{d}**
15	300	08.14±0.28	07.91± 0.41	07.84± 0.44	05.67±0.23	07.39±0.27^{b}
	100	09.81±0.42	12.52± 0.48	12.73 ±0.43	12.92±0.50	12.00±0.43^{a}
Means		**08.98±0.33^{b}**	**10.22±0.42^{a}**	**10.29±0.42^{a}**	**09.30±0.32^{b}**	**09.70±0.35 ^{d}**
30	300	08.81±0.48	08.45±0.50	08.32 ±0.42	05.61±0.34	07.80±0.41^{b}
	100	12.72±0.54	17.69 ±1.01	23.15 ±1.12	25.31± 1.16	19.72±0.84^{a}
Means		**10.77±0.46^{c}**	**13.07±0.71^{b}**	**15.74±0.87^{a}**	**15.46±0.76^{a}**	**13.76±0.77 ^{c}**
45	300	09.14±0.55	08.72±0.63	08.57± 0.57	05.77±0.49	08.05± 0.57^{b}
	100	17.97±1.11	27.61±1.54	35.33±1.21	39.97±1.55	30.22±1.01^{a}
Means		**13.56±0.81^{c}**	**18.17±0.89^{b}**	**21.95±0.93^{a}**	**22.87±1.21^{a}**	**19.14±0.96^{b}**
60	300	09.82±0.68	09.37±0.54	08.92±0.49	05.89±0.41	08.50±0.47^{b}
	100	25.02±.37	39.31±1.85	50.15±2.77	57.31± 2.83	42.95±1.91^{a}
Means		**17.42±1.14^{c}**	**24.34±1.37^{b}**	**29.54±1.74^{a}**	**31.60±1.98^{a}**	**25.73±1.51^{a}**
Overall means		**11.95±0.54 ^{c}**	**15.13± 0.73^{b}**	**17.49± 0.81^{a}**	**17.62± 0.96^{a}**	**15.55±0.69**

Means within the same row (a, b, c) or within the same column (A, B, C, D) bearing different letter superscripts are significantly ($P \leq 0.05$ or 0.01) different.

Table 5. Percentages of spermatozoa with coiled tails of Hy-Plus rabbit semen diluted with different levels of Doum as responses to hypo-osmotic solution, during incubation at 37 ^{o}C for up to 60 minutes (Means ± SE).

Incubation time (Minutes)	Osmolarities (mOsm/L)	Level of aqueous extracts of Doum (ml/ 100 ml diluted semen)				Overall means
		0.0 (Control)	1 (T_1)	2 (T_2)	3 (T_3)	
0	300	05.88±0.29	03.61 ± 0.26	02.71 ± 0.16	01.84±0.18	03.51± 0.15b
	100	10.14±0.33	12.54 ± 0.59	15.81 ± 0.68	20.77± 0.81	14.82± 0.41^{a}
Means		**8.01±0.26^{c}**	**8.08±0.31^{c}**	**9.26± 0.42^{b}**	**11.31±0.43^{a}**	**09.16±0.29^{e}**
15	300	06.18±0.29	03.89 ± 0.30	02.88 ± 0.24	02.21±0.21	03.79± 0.18^{b}
	100	15.55±0.62	20.00 ± 0.69	24.72 ± 1.12	31.52±1.25	22.95± 0.84^{a}
Means		**10.87±0.31^{d}**	**11.95±0.29^{c}**	**13.80±0.57^{b}**	**16.87±0.76^{a}**	**13.37±0.42^{d}**
30	300	06.61 ± 0.37	03.82 ± 0.41	02.91±0.27	02.16±0.31	3.88± 0.26^{b}
	100	25.37±1.54	31.88±1.85	38.94±2.13	46.57±2.81	35.69± 1.25^{a}
Means		**15.99±0.52^{d}**	**17.85±0.61^{c}**	**20.93±0.79^{b}**	**24.37±0.83^{a}**	**19.79± 0.72^{c}**
45	300	07.81± 0.66	05.11 ± 0.71	04.21 ± 0.69	03.27±0.51	05.10± 0.37^{b}
	100	35.41±1.67	44.16 ± 2.01	53.82 ± 2.21	62.15± 2.37	48.89± 1.92^{a}
Means		**21.61±0.94^{d}**	**24.64±1.37^{c}**	**29.02±1.44^{b}**	**32.71±1.29^{a}**	**27.00±1.15^{b}**
60	300	08.52±0.83	05.02±0.77	04.79 ±0.64	04.01±0.61	05.59±0.63^{b}
	100	41.12±2.31	63.51±2.91	71.24±3.21	83.14±3.64	64.75±2.47^{a}
Means		**24.82±0.87^{d}**	**34.27±1.22^{c}**	**38.04±1.47^{b}**	**43.58±1.41^{a}**	**35.17±1.19^{a}**
Overall means		**16.26±0.61^{d}**	**19.36± 0.73^{c}**	**22.21± 0.82^{b}**	**25.77± 0.78^{a}**	**20.90±0.84**

Means within the same row (a, b, c, d) or within the same column (A, B, C, D, E) bearing different letter superscripts are significantly ($P \leq 0.05$ or 0.01) different.

Percentages of swollen spermatozoa and spermatozoa with coiled tails of the four rabbit semen samples used were significantly ($P \leq 0.01$) higher in hypo-osmotic solution than normal osmotic solution. These results are in agreement with those obtained by Daader and Seleem (2005) and Zeidan *et al.* (2005). These results may be due to that spermatozoa exhibited morphologic changes which were evidenced by coiling of the tail, when subjected to HOS-test as shown in Figure 1 (Zeidan *et al.*, 2005).

The advancement of incubation time at 37 ^{0}C for up to 60 minutes increased significantly ($P \leq 0.01$) the percentages of swollen spermatozoa and spermatozoa with coiled tails in all rabbit semen studied under both osmolarities used. These results are in agreement with those obtained by Daader and Seleem (2005) and Zeidan *et al.*, (2005). Adding 3 ml aqua's extract/ 100 ml diluted semen insignificantly increased grade and percentages of sperm motility and spermatozoa with swollen head, compared with 2 ml of the extract.

Regarding different times of semen preservation at incubation condition (15, 30, 45 and 60 minutes), percentages of spermatozoa with

coiled tails or swollen heads in solution at 300 mOsm/L were significantly ($P \leq 0.01$) lower than those in solution at 100 mOsm/L.

The response of spermatozoa to hypo-osmotic solution may be due to the transportation of the physical and biochemical compounds across the sperm-cell membrane which plays an essential role in biochemical process and consequently sperm viability and fertilizing capabilities (Zavos, 1983). In addition, Daader and Seleem (2005) and Zeidan *et al.* (2005) added that, the abrupt decrease in osmotic pressure may cause malfunction in physiological processes of spermatozoa.

It's apparent clearly from these results that, the semen characters of Rabbit semen treated with Doum is better than control.

The results confirmed that, Doum is a good antioxidants with strong 1, 1 diphenyl-2-picrylehydrozyl (DPPH) radicals-scavenging activity, as well as, the investigated extracts had good antimicrobial activity, especially against bacteria (Hussein *et al*., 1998, Sanchez-Mareno *et al*., 1999 ; Dawidowicz *et al*., 2006). Plants are known to produce certain chemicals, which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria (Pesewu *et al*., 2008 ; Tasdelen *et al.*, 2009). Detailed study of the photochemistry of fruits and vegetable provides insight about phenolic compounds (Giugliano, 2000, Katsube *et al*., 2004 ; Dimitrios, 2006). These phenolic compounds often exhibit a wide range of physiological activities that include antioxidant, antimutagenic, anticarcinogenic, antimicrobial, and anti-inflammatory properties (Baliga and Katiyar, 2006 and Heinonen, 2007). Some information relate to the anti-nutritional factors such as of phytate, saponin and tannin. It is possible that they can occur in tea infusions from doum (Umaru *et al.*, 2007).

It could be concluded that, the response of rabbit spermatozoa to hypo-osmotic swollen test (HOS-test) was good indicator for reproductive capability of rabbit bucks as the hypo-osmotic condition that spermatozoa are subjected is considered as stressful factor, under which spermatozoa that show better results of motility or survivability can be used as a good indicator of fertilizing ability. In such a case it is not necessary to carry out more than one test for better evaluation of spermatozoa.

In conclusion, adding aqueous extracts of Doum improved diluted rabbit semen quality, during incubation condition. From economical point, 3 ml aqueous extracts of Doum / 100 ml diluted semen is sufficient.

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تأثير الدوم على استجابة الحيوانات المنوية للأرانب لإختبار إنخفاض الأسموزية

صفاء عطايا بركات* - رويدا محمد رياض**
* معهد بحوث الإنتاج الحيوانى، مركز البحوث الزراعية، الدقى، جيزة، مصر.
** معهد بحوث التناسليات الحيوانية، مركز البحوث الزراعية، جيزة، مصر.

أستخدم فى هذه الدراسة عدد ١٨ ذكر أرنب هاى بلس ناضج جنسياً عمر ٩ أشهر. صممت الدراسة لتقييم الدرجة والنسبة المئوية للحركة التقدمية للحيوانات المنوية بالإضافة إلى النسبة المئوية للحيوانات المنوية ذات الذيول الملتوية أو الرؤوس المنتفخة للسائل المنوى للأرانب المخفف والمضاف إليه مستويات مختلفة من المستخلص المائى لثمار الدوم لتقدير إستجابة الحيوانات المنوية للمحلول منخفض الأسموزية، خلال الحفظ على درجة حرارة التحضين (٣٧ °م) لمدة ٦٠ دقيقه. تم جمع السائل المنوى إصطناعياً، والقذفات ذات الحركة التقدمية للحيوانات المنوية أقل من ٧٠% تم استبعادها، عينات السائل المنوى المقبولة كانت تخلط وتقسم إلى جزئين متجانسين، الجزء الأول كان يخفف على درجة أسموزية طبيعية (٣٠٠ مل أسمول/ لتر، أما الجزء الثانى فكان يخفف باستخدام منخفض الأسموزية، أو ذات الإجهاد الإسموزى المنخفض (١٠٠ مل أسمول/ لتر)، بعد ذلك كان يقسم كل جزء من الجزئين الأساسيين للسائل المنوى المخفف إلى أربعة أجزاء أخرى تجريبية متجانسة، وكان يترك الجزء الأول بدون معاملة ويستخدم كمقارنة، أما الأجزاء الثلاثة الأخرى فكان يضاف إليهما المستخلص المائى لثمار نبات الدوم بمستويات ١، ٢، ٣ مل/ ١٠٠ مل سائل منوى مخفف، على الترتيب. بعد ذلك تم حفظ كل عينات السائل المنوى على درجة حرارة التحضين (٣٧ °م) لمدة ٦٠ دقيقه.

أوضحت النتائج المتحصل عليها من هذه الدراسة أن حركة الحيوانات المنوية فى المحاليل مختلفة الإسموزية (٣٠٠ أو ١٠٠ مل أسمول/ لنر) كانت أعلى معنوياً (على مستوى ١%)، بينما سجلت النسبة المئوية للحيوانات للمنوية ذات الذيول الملتوية أو الرؤوس المنتفخة إنخفاضاً معنوياً (على مستوى ١%) والمحفوظة فى المخفف ٣٠٠ مل أسمول/ لتر (الإسموزية الطبيعية)، مقارنة بتلك المحفوظة فى المخفف منخفض الأسموزية ١٠٠ مل أسمول/ لتر (إجهاد منخفض الأسموزية) خلال فترات الحفظ على درجة ٣٧ °م، ولمدة ٦٠ دقيقة فى كل المعاملات التجريبية المدروسة.

بتقدم زمن حفظ السائل المنوى المخفف على درجة حرارة التحضين (٣٧ °م) حتى ٦٠ دقيقة فى المحاليل الإسموزية المختلفة الاسموزية على الترتيب أدى إلى إنخفاض معنوى (على مستوى ١%) فى درجة والنسبة المئوية للحركة التقدمية للحيوانات المنوية، فى حين أن النسبة المئوية للحيوانات المنوية ذات الرؤوس المنتفخة أو الذيول الملتوية زادت معنوياً (على مستوى ١%)، فى كل مجاميع السائل المنوى المدروسة. درجة حركة الحيوانات المنوية والنسب المئوية لكل من الحركة التقدمية للحيوانات المنوية، الحيوانات المنوية ذات الرؤوس المنتفخة أو الذيول الملتوية فى كلا المحاليل الإسموزية المختلفة والمستخدمة (٣٠٠ أو ١٠٠ مل أسمول/ لتر) كانت مرتبة معنوياً (على مستوى ١%) ترتيباً تنازلياً، للسائل المنوى المخفف والمضاف إلية مستخلص نبات الدوم بمستويات ٣، ٢، ١ مل/ ١٠٠ مل سائل منوى مخفف، على الترتيب، مقارنة بالكنترول. إضافة ٣ مل مستخلص ثمار نبات الدوم لكل ١٠٠ مل سائل منوى مخفف أدى إلى زيادة غير معنوية فى درجة حركة الحيوانات المنوية والنسب المئوية للحركة التقدمية للحيوانات المنوية، وكذلك الحيوانات المنوية ذات الرؤوس المنتفخة، مقارنة بـ ٢ مل إضافة من المستخلص.

التوصية: نستخلص أن إضافة المستخلص المائى لثمار نبات الدوم إلى سائل منوى الأرانب المخفف حسن من جودته، وأطال القدرة التخزينية للسائل المنوى للأرانب، خلال الحفظ على درجة حرارة التحضين (٣٧ °م).